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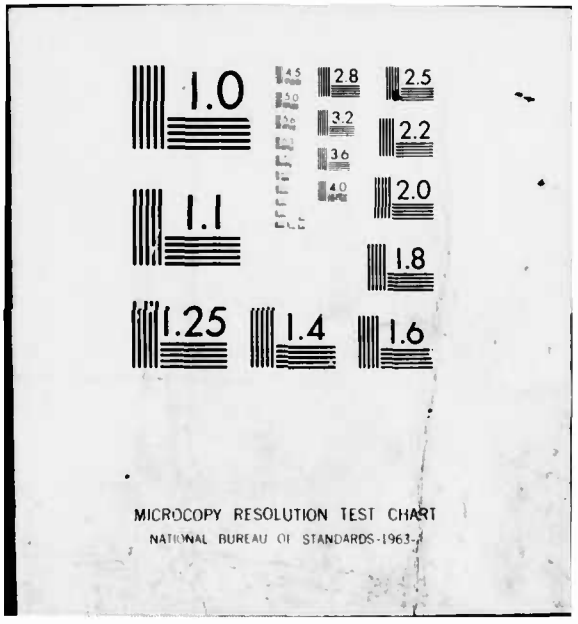
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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER (6)

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER (9) Rept. for

4. TITLE (and Subtitle)

Healing of the Oral Mucosa with the Use of Collagen Artificial Skin.

5. TYPE OF REPORT & PERIOD COVERED

Manuscript
10 Nov 1975 - 15 Feb 1977

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s) (10)

Marvin P. Levin, Peter J. Tsaknis
Duane E. Cutright

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS

U.S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D.C. 20012

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS (11)

U.S. Army Medical Research & Development Command
HQDA (SGRD-PL)
WASH DC 20314

12. REPORT DATE

30 March 1978

13. NUMBER OF PAGES

19

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

(12) 19p.

15. SECURITY CLASS. (of this report)

UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

DDC RECEIVED JUN 12 1978 F

18. SUPPLEMENTARY NOTES

None

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

healing, collagen, artificial skin, oral mucosa, biomaterials, wound dressing, gingival dressing.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This study utilized an enzyme solubilized calfskin collagen as a dressing for mucosal and gingival wounds in rabbits and dogs. Clinical and histological evaluations carried through the 14 day duration of the experiment showed similar healing between experimental and control sites. The experimental side healed slightly faster and the pigmented tissue was more uniformly regenerated when compared to the controls. The advantages of this wound covering are discussed.

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HEALING OF THE ORAL MUCOSA WITH THE USE OF COLLAGEN ARTIFICIAL SKIN

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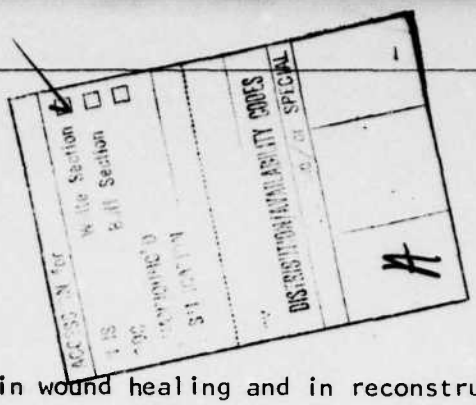

INTRODUCTION

The use of biomaterials to aid in wound healing and in reconstructive surgery has intriguing potential. If a surgeon can take a biologically derived material from a pre-sterilized package and use it as a graft instead of removing tissue from another area on the patient, he can minimize the surgery time and eliminate postoperative problems at the donor side. There would be a benefit to both the patient and the surgeon.

In the past few years several articles have appeared in the literature describing investigations with modified collagen membranes.[1-3] Successful results have been described in dialysis and corneal implants.[4,5] The purpose of the present study is to determine the biocompatibility of collagen artificial skin in the oral mucosa.

MATERIALS AND METHODS

The material used in this investigation is an enzyme-solubilized calfskin collagen originally developed by Nishihara and modified at the Rogosin Laboratories, Cornell University Medical College.[4] It is packaged individually in sheets 10 x 10 cm and can easily be cut with a scissors to any shape or size desired.

In the first part of the study, 15 New Zealand white rabbits were used. A recipient site was prepared by scalpel blade excision in the muco-buccal vestibule on the maxillae of each animal. A section approximately 2 x 2 cm consisting of epithelium, connective tissue, and underlying subcutaneous fat was removed. (Fig. 1) Periosteum was left intact.

A piece of Nippi Collagen Artificial Skin*, which was previously sterilized with ethylene oxide, was cut to size and sutured over the prepared wound. (Figure 2) An identical wound was made contralaterally, but no covering was placed; this served as a control.

The rabbits were sacrificed according to a time schedule that rendered three surgical sites and three controls for 3, 5, 10, 14, and 21 days. Specimens were harvested, fixed in 10% buffered formalin, imbedded in paraffin, and stained with hematoxylin and eosin.

As a second part of the study, surgical procedures were performed in 5 dogs on the facial maxillary and mandibular attached gingivae and alveolar mucosae of all four canine teeth. The wounds resembled recipient sites for free tissue autografts. (Figure 3) Collagen was sutured over the left maxillary and mandibular wound areas. (Figure 4) The right side served as the control. Gross and histological evaluations were made on 5, 7, and 14 day specimens.

RESULTS

The rabbit findings showed that experimental and control sites were almost identical. Observation of the surgical site at 3 and 5 days after surgery revealed a wound covered by a white opaque coagulum of soft tissue. Attempts at removing the granulation tissue plug indicated the wound was well organized and firmly established to the underlying and surrounding mucosa. The mucosal periphery assumed a normal light pink color and there was no evidence of edema in the area.

* Japan Leather Company, Ltd., Tokyo, Japan

Microscopically the specimens displayed all the elements of normally healing mucosal granulation tissue. Proliferating blood vessels were universal throughout the wounds. The predominant cellular component consisted of polymorphonuclear leukocytes, which were particularly heavy at the superficial aspects of the wound. Lymphocytes and plasma cells were noted but were much fewer in number, and eosinophils and foreign body giant cells were not seen. Epithelial resurfacing was actively advancing and extended below the upper necrotic portion of the wound but above the underlying proliferating and organizing granulation tissue. (Figure 5a)

Succeeding time periods of 10, 14, and 21 days demonstrated a subsequent reduction of the inflammatory cells and of the vascularity. A continuous contraction of the wound occurred with formation of mature fibrous connective tissue, eventual total re-epithelialization and complete unremarkable healing. (Figure 5b) At the final sacrifice period of 21 days, the original wound site was indiscernible. The results, therefore, indicated that the collagen graft did not cause any adverse reaction to wound healing in the oral mucosae of rabbits.

In dogs the artificial skin was seen to readily adhere to the wound sites and acted as a hemostatis agent. (Figure 4) At 5 days postoperatively adhering,organizing granulation tissue similarly covered the entire surgical wound in both experimental and control animals. Edema, although present, was not pronounced.

By the 7th day inflammation was diminishing but was still present. Clinically, healing was slightly more advanced in the experimental animals

and there was evidence of some pigmentation on the epithelial surface. (Figures 6a, 6b, and 6c) The 14-day postoperative sites showed the final stages of epithelialization. (Figures 7a, 7b, and 7c) There was no indication of abnormal mitosis, nuclear pleomorphism, or dysplastic changes in either the control or experimental specimens.

DISCUSSION

Within the parameters of this study, results indicate the modified collagen graft does not effect an adverse reaction in any of the animals, rabbit or dog, when placed as a wound covering. The dog gingivae did heal slightly more rapidly on the experimental side. The authors are aware that many variables exist in an investigation such as this, and conclusions must be tempered. There is no indication of what would occur if the material were to be placed over an oral wound in a human. Studies have already shown that, when the collagen is treated by ultraviolet radiation, immunologic reactivity is lost.[3,7] Thus, antigenicity may not be a problem and was not detected in this study.

In the present investigation there is no means of determining if the collagen membrane actually entered into the healing process, contributing its triple helix structure as a latticework for regenerating tissue. Or does it function strictly as a biologic dressing? In either case the collagen dressing certainly does not appear to delay healing. The advantages of collagen membranes are comparable to those ascribed to freeze-dried skin grafts,[8] namely:

1. Protection of the surgical wound from irritation and trauma.

2. Elimination of a donor site markedly reduces patient morbidity.

3. Abundant availability of graft material does not impose dimensional limitations to the surgical site.

The utilization of collagen membranes in oral surgery and periodontics offers various potential ideas. Additional research is much needed in this area.

SUMMARY

Wounds were prepared in the oral cavity of 15 rabbits and 5 dogs, and an enzyme-solubilized calfskin collagen was placed over the surgery sites on one side. The contralateral sides acted as controls. Results indicated the membrane is biologically acceptable to the oral mucosae of rabbits and dogs. The collagen did not cause any adverse reactions and may have been responsible for the clinical opinion of slightly more rapid healing of the gingivae in dogs. Clinical implications of this material's utilization produce some exciting ideas for future research.

This paper was presented in part at the 55th general meeting of the International Association for Dental Research, April 1977, Copenhagen.

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LEGENDS

Figure 1. Surgical site in muco-buccal vestibule of rabbit.

Figure 2. Collagen membrane sutured to prepared surgical site. Note hemostatic effect of the membrane.

Figure 3. Surgical site prepared on maxillary left facial aspect of dog canine.

Figure 4. Collagen membrane sutured over surgical area. Note hemostasis.

Figure 5a. 5-day rabbit, experimental side. Actively regenerating stratified squamous epithelium displaces necrotizing granulated plug (64X).

Figure 6a. 7-day post op, dog, experimental side. Note beginning of pigmentation (arrow).

Figure 6b. 7-day post-op, dog, control side.

Figure 6c. 7-day dog, experimental side. Peripheral migration of proliferating epithelium across the surgical wound is apparent. Note paucity of inflammatory cells and pronounced vascularity (64X).

Figure 7a. 14-day post-op, dog, experimental side. There is good healing from the surgery, but a marginal gingivitis is present.

Figure 7b. 14-day post-op, dog, control side. There is good healing but a marginal gingivitis is present. Pigmentation is not complete.

Figure 7c. 14-day dog, experimental side. Resurfacing by parakeratinized stratified squamous epithelium is complete (64X).

ACKNOWLEDGEMENTS:
The authors are grateful for the technical assistance provided by Mr. Eugene Peguese and Ms. Janet Pierce.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5a

FIGURE 6a

FIGURE 6b

FIGURE 7a

FIGURE 7b

FIGURE 7c